Epigenetics 101

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Epigenetics investigates stable, potentially heritable changes in gene expression that occur without changes in DNA sequence. Several epigenetic mechanisms, including DNA methylation, histone modifications, and miRNAs can change genome function under exogenous influence. Results obtained from animal models indicate that in utero or early-life environmental exposures produce effects on the epigenome that can have lifelong consequences and even be inherited trans-generationally. The search for human equivalents of the epigenetic mechanisms identified in animal models is in progress. I will present evidence from human environmental studies indicating that epigenetic alterations may mediate, or at least help to predict, effects caused by toxic exposures. In these investigations, we have shown that exposures, including air pollution, lead, arsenic, nickel, and PAHs, are associated with altered methylation of human repetitive elements or genes. In recent preliminary studies, we have shown alterations of histone modifications and miRNAs in subjects exposed to metal-rich airborne particles. I will present original data demonstrating that altered DNA methylation in blood and other tissues is associated with potentially related disease, such as cardiovascular disease, obesity, and asthma. On the basis of current evidence, I will propose possible models for the interplay between toxicants and the human epigenome.

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Andrea Baccarelli, MD, PhD, MPH, is the Mark and Catherine Winkler Associate Professor of Environmental Epidemiology in the Department of Environmental Health at the Harvard T.H. Chan School of Public Health. Dr. Baccarelli's research focuses on epigenomics as a unique molecular substrate reflecting the impact of environmental exposures on human health. Dr. Baccarelli's Human Laboratory of Environmental Epigenomics is dedicated to the investigation of environmental epigenetics at different life-stages. His ongoing projects range from the investigation of the effects of in-utero exposures to toxic metals, second-hand smoking, and psychosocial stress on the methylome of human fetal tissues to the study of the influences of air pollution on non-coding miRNA in adult and elderly individuals. Epigenetic mechanisms are investigated in relation to fetal growth and perinatal outcomes, cardiovascular function, obesity, and neuro-cognition. Since 2010, Dr. Baccarelli's laboratory has produced more than 130 peer-reviewed publications in epigenetics, environmental health, and epidemiology. Dr. Baccarelli earned his MD from the University of Perugia, Italy, his PhD from the University of Milan, Italy, and his MPH from the University of Turin, Italy. He completed his residency (Endocrinology) at the University of Milan, and a postdoctoral fellowship at the National Cancer Institute's Division of Cancer Epidemiology and Genetics.

Dr. Andrea Baccarelli

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Curriculum Vitae

Personal information

First name(s) / Surname(s) **Giorgio Assennato M.D., Sc.D., M.P.H.**

[redacted]

Work experience

Dates	From March 2006 till present
Occupation or position held	General Director of Apulian Environmental Prevention and Protection Agency
Main activities and responsibilities	The General Director is the legal representative of ARPA and takes all steps necessary to ensure its proper management. In particular, coordinates all functions necessary for the management, implementation and organization of institutional tasks
Name and address of employer	ARPA Puglia , Corso Trieste 27, 70126 Bari ,Italy
Type of business or sector	Apulia Regional Environmental Prevention and Protection Agency
Dates	From 21.02.2011 till present
Occupation or position held	President AssoArpa
Main activities and responsibilities	A non profit Association for achieving high levels of integration and policy development within all agencies associated, in matters of institutional relations and activities , governance and human resource, promote initiatives with particular reference to reviews of corporate policies for primary prevention and environmental monitoring as well as the definition of financing systems- to promote initiatives of study and / or research in the areas covered by the association's goals, analysis and proposal.
Name and address of employer	ASSO ARPA Association
Type of business or sector	Environmental prevention and protection association
Dates	2003
Occupation or position held	Consultant
Main activities and responsibilities	Consultant for the preparation of a Master's program in Environmental and Occupational Health per l'Adrea Stampar School of Public Health di Zagabria, Croazia
Name and address of employer	Council of Europe
Type of business or sector	Education
Dates	From 2002 till 2011
Occupation or position held	Full Professor of Occupational Medicine, School of Medicine,University of Bari
Main activities and responsibilities	
Name and address of employer	University of Bari, Italy
Type of business or sector	Department Internal and Public Medicine

Dates	1998
Occupation or position held	Consultant
Main activities and responsibilities	Adviser training program on the effects of chemicals exposure
Name and address of employer	Institute of Chemical Defence, Chemical Casuality
Type of business or sector	U.S. Army Medical Research

Dates	1997- 2003
Occupation or position held	Director
Main activities and responsibilities	Director of the School of Occupational Medicine
Name and address of employer	School of Occupational Medicine
Type of business or sector	University Of Bari

Dates	1992- 1997
Occupation or position held	Rector Commissioner for University of Bari
Main activities and responsibilities	He was appointed in special commission to examine various aspects of management related to University Libraries users and to support the Committee for Education , Training and Research
Name and address of employer	University of Bari
Type of business or sector	University

Dates	1989 -1997
Occupation or position held	Member
Main activities and responsibilities	Promoter of agreements with the University of Johns Hopkins University in Baltimore, USA, Ben Gurion University of the Negev, Beer-Sheva, Israel, and the University Tirana, international collaboration with scientists and foreign professors for planning post graduate training courses, seminars and joint research.
Name and address of employer	University of Bari
Type of business or sector	University

Dates	1988 to 2005
Occupation or position held	Head Physician
Main activities and responsibilities	Chief of the Service of Epidemiology
Name and address of employer	Clinica del Lavoro di Pavia, sede di Bari
Type of business or sector	

Dates	1987-1992
Occupation or position held	Advicer Board of Directors
Main activities and responsibilities	Advicer Board of Directors University of Bari as representative of associate professor
Name and address of employer	Università of Bari
Type of business or sector	

Dates	From April to May 1985
Occupation or position held	Visiting Professor
Main activities and responsibilities	Epidemiology Professor
Name and address of employer	Johns Hopkins University of Baltimora
Type of business or sector	School of Hygiene and Public Heath, Division of Occupational Medicine

Dates	1985
Occupation or position held	Chief of Epidemiology
Main activities and responsibilities	Responsible for epidemiological follow-up study of cloracneici in Seveso, on behalf of the Lombardy Regiont
Name and address of employer	Lombardy Region
Type of business or sector	Research

Dates	From 1979 to 1983
Occupation or position held	Consultant
Main activities and responsibilities	Scientific Cancer Institute of Genoa for Epidemiological Occupational Medicine studies of workers exposed to TCDD in the Seveso area
Name and address of employer	Cancer Scientific Institute of Genoa
Type of business or sector	Research

Education and training

Dates	1979-1983
Title of qualification awarded	Doctor of Science
Principal subjects/occupational skills covered	Master of Public Health, Division of Occupational Medicine
Name and type of organisation providing education and training	Johns Hopkins University, School of Hygiene and Public Health, Baltimore-USA

Dates	1977-1978
Title of qualification awarded	Master of Public Health (M.P.H.)
Principal subjects/occupational skills covered	Master of Public Health, Division of Occupational Medicine
Name and type of organisation providing education and training	Johns Hopkins University, School of Hygiene and Public Health, Baltimore USA

Dates	1975
Title of qualification awarded	
Principal subjects/occupational skills covered	Social Medicine
Name and type of organisation providing education and training)	University of Rome, Italy

Dates	1974
Principal subjects/occupational skills covered	Course in social medicine culture
Name and type of organisation providing education and training	University of Pisa, Italy
Dates	1973
Principal subjects/occupational skills covered	Course in Toxicology
Name and type of organisation providing education and training	University of Pavia, Italy
Dates	1972-1974
Title of qualification awarded	Specialist in occupational medicine
Name and type of organisation providing education and training	University of Turin, Italy
Dates	1972
Title of qualification awarded	Medical Degree
Name and type of organisation providing education and training	University of Bari, Italy
Mother Language	Italian
Other language	English
Additional Information	Since July 2007, Member of the Scientific Committee ISPESL, as expert appointed by the President. -Member of the Technical Operational Epidemiological Regional Observatory - Apulia Region -Scientific Director of the Centre of Research Epidemiologicals Regional Project - Apulia Region in Plans clean-up of areas with a high risk of environmental crisis of Taranto and Brindisi (Presidential Decree 23 April 1998, OJ 196 of 30 November 1998) -Scientific Director of projects for the Ministry of Health ex art.12 -Scientific Responsible of projects MIUR-PRIN as coordinator national and Head of Unit -Scientific Director of research projects in collaboration with ISPESL -Scientific Director of research projects in collaboration with the INAIL and INPS -Member of the UEMS (European Union of Medical Specialists) – Section Occupational Medicine -Member of the International Commission on Occupational Health (ICOH) -Member of the Italian Association of Epidemiology -Author of over 252 publications and communications to national and international congresses

Bari, 17.07.2015

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2014

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1980

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Bari, 17.07.2015

NCEA/NERL Workshop

Applying Epigenetic Data in Cumulative Risk Assessment
September, 2 and 3, 2015. Potomac Yards Conference Facility, Arlington, VA

This workshop focuses on the rapidly emerging role of epigenetic alterations as mediators that are:

- Associated with many chronic and developmental diseases; and
- Responsive to multiple environmental stressors.

Human disease is necessarily understood as driven by combination of genetic and environmental factors: Much research indicates that genetics by itself is not an effective predictive of disease. And contributing environmental factors are necessarily broadly defined, including dietary imbalance, environmental pollution, effects of economic deprivation, and psychosocial stress.

Epigenetic changes are specific molecular changes around DNA that alter expression of genes. Epigenetic changes include DNA methylation, formation of histone adducts, and changes in micro RNAs. Research today indicates that are involved in many chronic diseases (cancer, cardiovascular disease, obesity, diabetes, mental health disorders, asthma). Research has also linked a wide range of stressors including pollution and social factors with occurrence of epigenetic alterations.

Only recently receiving attention is the nexus between the factors of cumulative exposure to environmental stress, epigenetic change, and the development of chronic disease. In the terminology of environmental science, epigenetic changes may be able to play a role as both biosensors of cumulative exposure and biomarkers of effect for disease processes.

[redacted]

This workshop will examine the concept of "epigenetic load" – accumulated epigenetic marks as influenced by multiple stressors - and how it can inform cumulative risk assessment. Important questions for understanding as the field develops include possible "tipping points" for cumulative epigenetic change, which when exceeded would compromise health. And whether, in a population already exposed to significant stressors, the addition of an additional stress (even if not large in magnitude) can lead to some increase in the probability of disease.

Specific Workshop goals:

- Examine current understanding of the role of epigenetic changes of multiple kinds in mediating between environmental stressors and chronic or developmental disease.
- Appraise the potential to use epigenetic change as a measure of cumulative, multi-stressor, exposure and impact.
- Define research and practical needs to advance epigenetics as a new tool for cumulative risk assessment.

CURRICULUM VITAE

NAME: Kamin James Johnson, PhD

CURRENT JOB TITLE: Lead Scientist, Developmental and Reproductive Toxicology; The Dow Chemical Company

EDUCATION:
1988-1994: Doctor of Philosophy, Brown University, Providence, RI
Major: Molecular Biology, Cell Biology, and Biochemistry

1984-1988: Bachelors of Science, University of Georgia, Athens, GA
Major: Genetics

EMPLOYMENT:
2013-Present: The Dow Chemical Company, Midland, MI; Lead Scientist, Toxicology and Environmental Research & Consulting, Developmental and Reproductive Toxicology

2007-2013: Alfred I. DuPont Hospital for Children, Wilmington, DE, Head, Perinatal Reproductive Malformations Lab

2003-2007: The Hamner Institutes for Health Sciences, Durham, NC, Assistant Investigator

2000-2003: Brown University, Providence, RI, Assistant Professor (Research), Department of Pathology and Laboratory Medicine

1998-2000**:** Brown University, Providence, RI, Postdoctoral Research Associate, Department of Pathology and Laboratory Medicine

1998-1998: Lund University, Lund, Sweden, Postdoctoral Research Associate, Department of Experimental Biology

1994-1998: Duke University, Durham, NC, Postdoctoral Research Associate, Department of Cell Biology

PUBLICATIONS:

Peer-Reviewed Articles:

Johnson, KJ, Hall, ES, and Boekelheide, K (1991). 2,5-Hexanedione exposure alters the rat Sertoli cell cytoskeleton. I. Microtubules and seminiferous tubule fluid secretion. *Toxicology and Applied Pharmacology* 111:432–442

Allard, E, Johnson, KJ and Boekelheide, K (1993). Colchicine disrupts the cytoskeleton of rat testis seminiferous epithelium in a stage-dependent manner. *Biology of Reproduction* 48:143–155.

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Lahousse, SA, Beall, S, and Johnson, KJ (2006). Mono-(2-ethylhexyl) phthalate rapidly increases celsr2 protein phosphorylation in HeLa cells via protein kinase C and casein kinase 1. *Toxicological Sciences* 91:255–264.

Lahousse, SA, Wallace, DG, Liu, D, Gaido, KW and Johnson, KJ (2006). Testicular gene expression profiling following prepubertal rat mono-(2-ethylhexyl) phthalate exposure suggests a common initial genetic response at fetal and prepubertal ages. *Toxicological Sciences* 93:369–381.

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Johnson, KJ, Hensley, JB, Kelso, MD, Wallace, DG, and Gaido, KW (2007). Gene expression changes in the fetal rat testis following acute dibutyl phthalate exposure defines a complex temporal cascade of responding cell types. *Biology of Reproduction* 77:978–989.

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Rolland, AD, Lehmann, KP, Johnson, KJ, Gaido KW, and Koopman, P (2011). Uncovering gene regulatory networks during mouse fetal germ cell development. *Biology of Reproduction* 84:790–800.

Johnson, KJ, McDowell, EN, Viereck, MP, and Xia, JQ (2011). Species-specific dibutyl phthalate fetal testis endocrine disruption correlates with inhibition of SREBP2-dependent gene expression pathways. *Toxicological Sciences* 120:460–474.

Heger, NE, Hall, SJ, Sandrof, MA, McDonnell, EV, Hensley, JB, McDowell, EN, Martin, KA, Gaido, KW, Johnson, KJ, and Boekelheide, K (2012). Human fetal testis xenografts are resistant to phthalate-induced endocrine disruption. *Environmental Health Perspectives* 120:1137-1143.

Johnson, KJ, Heger, NE, and Boekelheide, K (2012). Of mice and men (and rats): phthalate-induced fetal testis endocrine disruption is species-dependent. *Toxicological Sciences* 129:235-248.

McDowell, EN, Kisielewski, AE, Pike, JW, Franco, HL, Yao, HH, and Johnson, KJ. (2012). A transcriptome-wide screen for mRNAs enriched in fetal Leydig cells: CRHR1 agonism stimulates rat and mouse fetal testis steroidogenesis. *PLoS One* 7(10):e47359.

Pike JW, McDowell, E, McCahan, SM, and Johnson, KJ. (2014). Identification of gene expression changes in postnatal rat foreskin after in utero anti-androgen exposure. *Reproductive Toxicology* 47:42-50.

Johnson, KJ. (2015). Testicular histopathology associated with disruption of the Sertoli cell cytoskeleton. *Spermatogenesis* in press.

Marshall, VA, Johnson, KJ, Moore, NP, Rasoulpour, RJ, Tornesi, B, and Carney, EW. (2015). Comparative Response of Rat and Rabbit Conceptuses *In Vitro* to Inhibitors of Histiotrophic Nutrition. *Birth Defects Research Part B: Developmental and Reproductive Toxicology* in press.

Book chapters:

Boekelheide, K, Johnson, KJ and Richburg, JH (2004). Sertoli Cell Toxicants. In: *Sertoli Cell Biology*, Academic Press, San Diego.

Rasoulpour, RJ, Marty, MS, Johnson, KJ, and Carney, EW (2010). Normal Development of the Male Reproductive System. In: *Reproductive Toxicology*, Informa Healthcare, New York.

Non-peer-reviewed articles:

Johnson, KJ, and Carney, EW. (2014). New Strategies for Developmental Toxicity Assessment based on Adverse Outcome Pathways. Altox.org. Reproductive and Developmental Toxicity. The Way Forward

PRESENTATIONS:

Society of Toxicology Northeast Chapter Annual Meeting, Avon, CT; 1999: The cellular "handshake" in rat testis: basic biology and potential role in germ cell survival

Society of Toxicology Annual Meeting, Symposium Presentation, Nashville, TN; 2002: Pursuit of the testis phthalate target: a novel cadherin is an early responder

University of Texas, Department of Pharmacology and Toxicology, Austin, TX; 2003: Testis cadherins and the mechanism of phthalate-induced testis injury

Phthalate Esters and Reproductive Health Workshop, Mello, France; 2004: Phthalate hypotheses: What are the proximal targets?

Eastman Chemical Company, Kingsport, TN; 2004: Identifying phthalate testicular targets

Environmental Protection Agency Reproductive Toxicology Section, Research Triangle Park, NC; 2005: Early molecular changes in the testis following pubertal phthalate exposure

North Carolina State University, Environmental and Molecular Toxicology Department Seminar Series, Raleigh, NC; 2005: Protocadherins in spermatogenesis and the mechanism of phthalate-induced testicular injury

Alfred I. duPont Hospital for Children, Biomedical Research Department Seminar Series, Wilmington, DE; 2006: Testicular molecules and cells targeted by endocrine-active phthalates

Society of Toxicology Annual Meeting, Platform Session on Mechanistic Insights for Reproductive Toxicology, Baltimore, MD; 2009: Genomic profile of rat placenta after gestational phthalate exposure

Brown University, Department of Pathobiology and Laboratory Medicine, Providence, RI; 2009: Endocrine disruptors and male reproductive development

Society of Toxicology Annual Meeting, Platform Session on New Insights into Male Reproductive Toxicology, Washington, DC; 2011: In utero DBP exposure concomitantly inhibits fetal testis steroidogenic and SREBP2-dependent cholesterologenic pathways

ILSI Health and Environmental Sciences Institute's Developmental and Reproductive Toxicology (DART) Technical Committee Testicular Toxicity Workshop on In Vitro Models, Washington, DC 2011: Comparing in vitro and in vivo fetal testis endocrine disruption

The Teratology Society Annual Meeting, Symposium Presentation, Baltimore, MD, 2012: In utero environmental influences on male reproductive disease

The Society of Toxicology Annual Meeting, Workshop Presentation, San Antonio, TX, 2013: Endocrine disruption mediated developmental toxicity in mice versus rats: Implications for humans

Dupont Chemical Company, Wilmington, NC, 2013: Species-dependence and mixture effects of phthalate-induced fetal testis endocrine disruption

Food and Drug Administraction, Center for Food Safety and Applied Nutrition**,** Laurel, MD, 2013: Species-dependence and mixture effects of phthalate-induced fetal testis endocrine disruption

ABSTRACTS:

Lahousse, SA, Beall, SA, and Johnson, KJ (2006). Phthalates rapidly increase celsr2 protein phosphorylation via PKC and CK1. (Presented at the G Protein-Coupled Receptors: Evolving Concepts and New Techniques Keystone Symposium).

Johnson, KJ, Wallace,D, and Lahousse, S (2006). Gene expression profiling suggests a *conserved* initial mechanism for fetal and pubertal phthalate testicular injury. (Presented at the Society of Toxicology Annual Meeting).

Johnson, KJ, Wallace, DG, Liu, D, Gaido, KW, and Lahousse, SA (2006). Gene profiling of fetal and pubertal rat testis following acute phthalate exposure suggests a conserved genetic response. (Presented at the Gordon Conference on Environmental Endocrine Disruptors).

Johnson, KJ, Hensley, J, Kelso, M, Wallace, DG, and Gaido, KW (2007). Mapping induced gene expression in the phthalate-exposed fetal rat testis. (Presented at the 4th Copenhagen Endocrine Disruptors Workshop).

Barthold, JS, Johnson, KJ, McCahan, S, Wang, Y, Robbins, A, Sol-Church, K, and Devoto M (2009). The Estrogen Receptor Beta (ERß) Gene Is Associated with Cryptorchidism in Rat and Man. (Presented at the American Academy of Pediatrics National Conference).

Barthold, JS, Chacko, J, Robbins, A, Wang, Y, McCahan, S, and Johnson, KJ (2009). Hormonal Regulation of Gene Expression in the Fetal Rat Gubernaculum. (Presented at the American Academy of Pediatrics National Conference).

Pike, J, McCahan, SM, and Johnson, KJ (2011). In Utero Exposure to Dibutyl Phthalate Alters Gene Expression Levels in the Fetal Rat Foreskin. (Presented at the Society of Toxicology Annual Meeting).

Johnson, KJ, McDowell, E, and Pike, J (2012). Undernutrition Inhibits Fetal Testis Steroidogenesis. (Presented at the Society of Toxicology Annual Meeting).

Barthold, JS, Wang, Y, Robbins, A, Pike, J, McDowell, E, Johnson, KJ, and McCahan, S. (2012). Transcriptome Analysis of the Fetal Gubernaculums Following DHT Exposure Identifies Common Androgen and Insulin-like 3 Targets. (Presented at the American Academy of Pediatrics National Conference).

Pike, J, McDowell, EN, and Johnson, KJ (2012). Fetal Growth Restriction Leads to Inhibition of Steroidogenesis. (Presented at the Society for the Study of Reproduction Annual Meeting).

Johnson, KJ, Pike, J, Kisielewski, A, and McDowell, EN (2013). Candidate Genes Controlling Mouse Leydig Cell Function. (Presented at the Endocrine Society Annual Meeting).

Pike, J, Kisielewski, A, McDowell, EN, and Johnson, KJ (2013). In Utero Growth Restriction and Dibutyl Phthalate Exposure Cooperatively Disrupt Steroidogenesis. (Presented at the Endocrine Society Annual Meeting).

McDowell, EN, Kisielewski, A, and Johnson, KJ (2013). Regulation of Candidate Genes on Mouse Leydig Cell Function. (Presented at the Society for the Study of Reproduction Annual Meeting)

Frances Champagne – Columbia University

Proposed for Day 1, Session 1: Social Aspects of Stress

Biosketch: Prof. Champagne's main research interest concerns how genetic and environmental factors interact to regulate maternal behavior, and how natural variations in this behavior can shape the behavioral development of offspring through epigenetic changes in gene expression in a brain region specific manner.

Research excerpts:

- Monk C, Spicer J, Champagne FA (2012) Linking prenatal maternal adversity to developmental outcomes in infants: The role of epigenetic pathways. Development & Psychopathology24(4): 1361-1376.
- Danchin E, Charmantier A, Champagne FA, Mesoudi A, Pujol B, Blanchet S. (2011) Beyond DNA: Integrating inclusive inheritance into an extended theory of evolution. Nature Reviews Genetics 12(7):475-86.
- Champagne FA (2008) Epigenetic mechanisms and the transgenerational effects of maternal care. Frontiers in Neuroendocrinology 29(3): 386-397.

Michael Skinner – Washington State University

Proposed for Day 1, Session 2: Overview of epigenetic roles in the etiology of selected major disease

Biosketch: Dr. Michael Skinner is a professor in the School of Biological Sciences at Washington State University. He did his B.S. in chemistry at Reed College in Portland Oregon, his Ph.D, in biochemistry at Washington State University and his Postdoctoral Fellowship at the C.H. Best Institute at the University of Toronto. He has been on the faculty of Vanderbilt University and the University of California at San Francisco. Dr. Skinner's research is focused on the investigation of gonadal growth and differentiation, with emphasis in the area of reproductive biology. His current research has demonstrated the ability of environmental toxicants to promote the epigenetic transgenerational inheritance of disease phenotypes due to abnormal germ line epigenetic programming in gonadal development. Dr. Skinner has over 250 peer reviewed publications and has given over 260 invited symposia, plenary lectures and university seminars.

Research excerpts:

- Skinner MK (2013) Review of: Epigenetics: A Reference Manual. Edited by Jeffrey M. Craig and Nicholas C. Wong. Norfolk (United Kingdom): Caister Academic Press. The Quarterly Review of Biology Vol. 88 pgs. 351-352.
- Skinner MK (2013) Review of: Epigenetics: The Ultimate Mystery of Inheritance. By Richard C. Francis. New York: W. W. Norton & Company. The Quarterly Review of Biology Vol. 88 pg. 351.
- Skinner MK (2014) Environmentally Induced Epigenetic Transgenerational Inheritance of Disease: Ancestral Ghosts in Your Genome. The Teratology Society 54th Annual Meeting. Birth Defects Research (Part A) 100(5): pg 372, #S16.